signal integration times can be increased over conventional frame imaging modes by a factor exceeding 1000 fold.

Bead Illumination

As previously discussed in conjunction with several embodiments of the present invention and as shown in FIGURES 3 and 4, several different illumination systems may be employed to illuminate the beads in flow. A standard approach involves illuminating the beads in flow with a light source providing light along a path oriented orthogonal to the spectral decomposition and imaging system. Alternative modes of illumination, such as those shown and FIGURES 3 and 4 and disclosed in above-referenced U.S. Patent No. 6,211,955, allow for the generation of bright field, dark field, phase contrast, fluorescence and EPI fluorescence imagery. U.S. patent application Serial No. 09/689,172, entitled "Multipass Cavity for Illumination and Excitation of Moving Objects," filed on October 11, 2000, discloses a method for illumination in which the number of photons incident on the beads may be increased by a factor of 10 or more.

The design of the illumination system also allows the use of pulsed light source or other strobed sources for high sensitivity fluorescence measurement without any need to strobe in synchrony with bead flow. A high aspect ratio also allows for highly efficient coupling of linear array diode illumination into the cuvette.

Bead Velocity Measurement

For the second and third pixelated detection techniques, wherein an accurate knowledge of the bead velocity is required, either a frequency domain velocity measurement (FDVM) technique or a time domain velocity measurement (TDVM) technique, both as disclosed in U.S. patent application No. 09/939,292, filed on August 24, 2001 and entitled "Measuring the Velocity of Small Moving Objects Such as Cells" can be employed. In FDVM, a large FOV is imaged onto a ruling of opaque and transparent bars. Motion of the objects within the FOV causes modulation of their intensity as they pass across the ruling. The modulation frequency is proportional to the velocity of the objects and can be determined using Fast Fourier Transform analysis.

Velocity can also be determined using two detectors in a conventional time-of-flight measurement scheme, though with very restricted throughput. However, time-of-flight measurements become more complex when throughput increases and the times of flight of multiple objects are measured simultaneously. Such systems can fail when correlation is lost between the entry and exit times of the objects in view. The time-of-flight system preferably used relies on an improved scheme wherein the waveforms produced by the entry and exit detectors are cross-correlated to detect phase changes that are indicative of changes in velocity.

Sample Handling

A standard pumping technique of the type used in prior art flow cytometers can be used to hydrodynamically focus the beads with micron-scale positional accuracy within the cuvette. Hydrodynamic focussing of the beads ensures they are located in or near the plane of focus of the optical system. Handling reporter labeled beads in suspension in the present invention eliminates the need to array beads in a monolayer on a substrate. When analyzing large samples containing a billion beads, the substrate methodology becomes impractical due to the number of substrates or the size of a single substrate required to carry a billion or more beads. A sample of four billion, 10 micron beads occupies a volume of as little as 4.0 cm^3 or 4.0 milliliters. By comparison, when arrayed in a perfect tightly packed monolayer, that same four billion bead sample would occupy a square area of over 24 inches on a side. Or, when produced in more manageable format such as a standard microscope slide, the four billion bead sample will occupy more than 1728 slides. Other advantages of handling beads in suspension include the ability to sort individual beads at high speed, the ability to collect bead images from multiple perspectives, and the ability to hydrodynamically focus beads into a tight focussable core near the focal plane of the collection system. To increase throughput in flow, the sample stream may have a high aspect ratio in the plane perpendicular to flow. This technique is discussed in U.S. Patent No. 5,422,712 "Apparatus for Measuring Fluorescent Spectra of Particles in a Flow" the specification and figures of which are specifically incorporated herein by reference for purposes of providing background information regarding the technique commonly referred to as broad flat flow. Broad flat flow allows objects to flow in a plane as opposed to single file through the flow cell. This increases throughput by allowing beads to flow more or less parallel to each other while maintaining a tight plane of focus. Those skilled in the art will appreciate that broad flat flow can easily be created using commercially available flow cells as shown in FIGURE 16B containing flow cell 290, with a cross section that is elongated in an axis perpendicular to both the flow and optical axes.

Spatial and Spectral Corrections to Pixelated Imagery

In the second through fifth embodiments, a spatial and spectral correction may optionally be applied to the signals coming off the pixelated detector(s) to improve the integrity of the decoding process. A method and apparatus for such a system is disclosed in US provisional patent application Serial No. 60/286,713 entitled "Method and Apparatus for Cross-Talk and Spatial Registration Correction for Multi-channel Imaging." In this scheme, spatial registration errors between channels on the detector, or spatial registration errors between detectors, is corrected to more accurately determine the origin of light projected upon the detector. Likewise, once a spatial

correction has been applied, a spectral correction is applied to remove any spectral cross-talk between channels or detectors to more accurately determine the spectral content of light projected onto each pixel on the detector(s).

All figures illustrating imaging systems in the present invention employ pixelated detectors. In some embodiments multiple images of reporter labeled carrier beads are projected upon a single detector as illustrated in FIGURE 11. In other embodiments such as that illustrated FIGURE 16A multiple detectors are employed, each containing a different imagery of the same reporter labeled carrier beads.

Ideally, each of the reporters or carriers being imaged by the present invention would deliver light comprised of wavelengths entirely bounded by the edges of the passband of a particular channel. In that case, each source type (bead fluorochrome) would appear in only one channel of a detector or on only one detector in the case of a multiple detector embodiment. However, in many cases the fluorochromes used in the reporters, carriers or binding signals span a range of wavelengths broader than the passband of the associated imaging filter. This is clearly illustrated in FIGURE 23, where emission spectra of several fluorochromes are superimposed over the passbands of several channels on a wavelength versus intensity plot. In this case, light from each source 201 will be received by two or three channels 202. The signal conveyed by each channel, then, is a composite of information from multiple sources. This can make reporter identification difficult, especially when reporters are intensity coded as well as spectrally coded. Therefore, the signals from the various detectors or channels must be operated on in a way to remove the crosstalk to accurately determine the spectral content and intensity for each reporter, carrier or binding signal

Fundamentally, to remove crosstalk a set of linear equations as shown below must be solved .

$$s_1 = \alpha_{11} m_1 + \alpha_{12} m_2 + \alpha_{13} m_3$$
$$s_2 = \alpha_{21} m_1 + \alpha_{22} m_2 + \alpha_{23} m_3$$
and
$$s_3 = \alpha_{31} m_1 + \alpha_{32} m_2 + \alpha_{33} m_3$$
where :
m_j = measurement from channel j,
s_i = a characteristic parameter of source i, and
α_{ij} = weighting coefficient, source i into channel j.

The weighting coefficients carry information about the sources and about the channels used to collect the measurements. The equations are solved using the methods of linear algebra, wherein the variables s_i, and m_j are treated as vectors and variable $\alpha_{i,j}$